Chapter 1
Cell and Molecular Biology of the Liver:

A1. Define major pathways and molecular participants in signal transduction in liver cells. New signaling pathways have been identified in liver during the past year. Signaling events generally begin with plasma membrane receptors, so receptor desensitization and resensitization are critical gatekeepers. Hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) redirects certain internalized G protein-coupled receptors back to the plasma membrane to prevent lysosomal degradation (Hanyaloglu AC. *EMBO Journal* 2005; 24:2265). Calcium is a versatile second messenger, but excessive increases can lead to cell death. Cyclophilin D forms the mitochondrial permeability transition pore that results in the death of hepatocytes damaged by excessive increases in calcium or by oxidative stress; consequently, mice lacking cyclophilin D are resistant to these forms of cell death (Baines CP. *Nature* 2005; 434:658). Polycystin-2 is a cholangiocyte protein defective in autosomal dominant polycystic kidney and liver disease and has been linked to regulation of secretion by cholangiocytes and cholangiocyte proliferation (Li X. *Nature Cell Biology* 2005; 7:1102) (10%).

A2. Elucidate the mechanisms of lipid metabolism and transport in liver as it relates to whole body lipid homeostasis. This is an area of active investigator-initiated research. In the past year, the scavenger receptor class B type I (SR-BI) has been shown to be a regulator of cholesterol efflux from macrophages for excretion by the liver into bile and feces (Zhang YZ. *J Clin Invest* 2005;115:2870) Furthermore, the ABCG1 and ABCA1 transporters act synergistically in mediating cholesterol efflux from macrophages and incorporation into HDL particles (Gelissen IC. *Arterioscler Thromb Vasc Biol* 2005; In press). Biliary cholesterol secretion is regulated largely through the ABCG5/G8 transporters, which in turn are regulated by the nuclear receptors LXR, FXR and PXR (Yu L. *J Biol Chem* 2005;280:8742). (20%)

A3. Determine how intra- and inter-cellular signals are integrated *in vivo* to regulate liver function. The importance of cross-talk between Kupffer cells and hepatocytes was shown in studies of site specific deletion of IκB kinase (IKKβ) activity (required for NF-κB activation) in mice. When IKKβ was deleted from hepatocytes only, chemical-induced hepatocarcinogenesis was enhanced; whereas when it was deleted from both hepatocytes and Kupffer cells, hepatocarcinogenesis was decreased (Maeda S. *Cell* 2005;121: 977). (10%)

B1. Elucidate physiological importance of liver plasma membrane transporters and mechanisms of action. The organic solute and drug membrane uptake transporters located in liver cell plasma membranes include NTCP, and several organic anion and cation transporters (OATPs and OCTs), while the export transporters include multiple ATP-binding cassette (ABC) proteins (BSEP, MDR1 and 2, MRP2, 3 and 6). These transporters can be regulated by the orphan nuclear receptors, but also by several hormones (prolactin, growth hormone) and inflammatory signals (TNF-α, IL-1β) (Wood M. *Mol Pharmacol* 2005;68:218; Geier A. *Am J Physiol GI* 2005;289:831). The binding of OATP1A1 to cell

surface membranes is mediated in part by the chaperone protein PDZK1 (Wang P. *J Biol Chem* 2005;280:30143). (10%)

B2. Develop cell culture model that reflects different liver cell interactions (e.g., hepatocyte with Kupffer cell, cholangiocyte, stellate cell, or endothelial cell). Several groups of investigators are working on developing methods for co-culture of hepatocytes with Kupffer cells, bone marrow stromal cells and biliary epithelial cells (Zinchenko YS. *J Biomed Mater Res A.* 2005;75:242; Auth MK. *Liver Transpl* 2005;11:410; Takeda M. *J Biosci Bioeng* 2005;100:77). (10%)

B3. Elucidate intra- and extra-cellular events that determine hepatocyte polarity. Recent studies have shown that polarization of hepatocytes and development of canaliculi require recruitment of rab11a and myosin Vb to intracellular membranes that contain apical ABC transporters and transcytotic markers, permitting their appropriate targeting (Wakabayashi Y. *PNAS* 2005;102:15087). Delivery of rab11a-myosin Va-containing membranes to the cell surface leads to their differentiation into a bile canaliculus. (10%)

C1. Elucidate major elements in process of transcellular vesicle trafficking in the hepatocyte. Flow cytometry and florescent labeling has been used to directly visualize the binding and movement of vesicles along microtubules towards apical and basolateral membranes in the hepatocyte (Murray JW. *Meth Enzymol* 2005;403:92). The factors that determine binding, motion and fission of vesicles to membranes are now being elucidated. (0%)

C2. Elucidate how cells interact with each other (e.g., via gap junctions, ECM, paracrine, and endocrine signaling). Caveolin regulates the organization and activity of signaling molecules concentrated in caveolae in the plasma membrane. Dynamin-2 interacts directly with caveolin-1 in hepatocytes and mediates the scission of caveolae from the plasma membrane (Yao Q. *J Mol Biol*. 2005;348:491). (10%)

C3. Develop knowledge base of normal liver proteome, including analysis of individual cell types, subcellular compartments, and changes along hepatic acinus. Research to characterize the proteomes of liver and biliary cell types is encouraged by the NIH through program announcements, such as "Proteomics: Diabetes, Obesity, and Endocrine, Digestive, Kidney, Urologic, and Hematologic Diseases" (PA-04-081). International research efforts in this area are being coordinated through the Human Liver Proteome Project (HLPP). The HLPP pilot phase (2004-2006) is ending. The data generated by worldwide participating laboratories will be publicly available in Summer 2006 and will be presented at that time in a special issue of *Proteomics*. A major effort to generate antibodies against all liver proteins is underway. (0%)

Figure 3. Estimated Progress on Cell and Molecular Biology Research Goals, 2005 (Year 1)